Secondary compounds in caterpillars of four moth families (Noctuoidea, Bombycoidea) are partly identical

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Summary. Semiquantitative data on secondary compounds in hemolymph and, where present, in exocrine gland secretions were determined for caterpillars of 17 species of moths from four families. Based on these data, similarities of the chemical bouquets in the larval body fluids were computed using various distance measures. Clustering of larval hemolymph compounds resulted in phenograms which arranged the species corresponding mainly to their taxonomic relationship and permitted to characterize taxa. Phenograms based on the secretion compounds of Saturniidae and Lymantriidae seemed to be influenced also by the potential enemies addressed by the respective secretions. In both cases, basically similar metabolic pathways of secondary compounds in the four families and presumably also in other moths are suggested.

Zusammenfassung. Halbquantitative Daten zu Sekundärstoffen in Hämolymphe und, wenn vorhanden, in exokrinen Drüsensekreten wurden für Raupen von 17 Nachtfalterarten aus vier Familien bestimmt. Auf Grundlage dieser Daten wurde die Ähnlichkeit der chemischen Bouquets in den larvalen Körperflüssigkeiten mittels verschiedener Distanzmaße berechnet. Die Gruppenbildung auf Grundlage der larvalen Hämolymphe erzeugte Phänogramme der Arten, welche hauptsächlich ihrer taxonomischen Verwandtschaft entsprachen und auch eine Charakterisierung von Taxa erlaubten. Phänogramme, die auf der Zusammensetzung der Wehrsekrete der Saturniidae und Lymantriidae basierten, schienen auch von ökologischen Aufgaben der jeweiligen Sekrete beeinflußt zu sein. In beiden Fällen ließ sich ein Hinweis auf grundsätzlich ähnliche Stoffwechselwege für Sekundärstoffe bei den vier Familien und vermutlich auch anderen Nachtfaltern erkennen.

Resumé. Des données sémi-quantitatives relatives aux composantes secondaires de l'hémolymphe et, quand présentes, dans les sécrétions de glandes exocrines, ont été déterminées pour les chenilles de 17 espèces de papillons nocturnes appartenant à quatre familles. Sur base de ces données, des similarités entre les bouquets chimiques des fluides corporels larvaires ont été computées au moyen de plusieurs mesures de distance. Le clustering des composantes de l'hémolymphe larvaire à résulté en des phénogrammes qui arrangent les espèces principalement selon leur parenté taxinomique, permettant ainsi de caractériser les taxons. Les phénogrammes basés sur les composantes de sécrétion des Saturniidae et des Lymantriidae paraissaient également être influencés par les ennemis potentiels interpellés par les sécrétions respectives. Dans les deux cas, des trajets métaboliques de composantes secondaires essentiellement similaires au sein des quatre familles, ainsi que vraisemblablement aussi auprès d'autres papillons nocturnes, sont suggérés.

Key words. Eupterotidae, exocrine secretions, hemolymph, Lepidoptera, Lymantriidae, Notodontidae, Saturniidae, secondary compounds.

Introduction

Chemical inventories of secondary compounds have been taken from a wealth of insect species. Such components which are often connected with chemical defence may be of interest for comparative physiological or biochemical purposes, in particular, if they refer to complex metabolic pathways and an according enzyme repertoire needed. However, few comparative efforts using such allomones have been undertaken hitherto in the Lepidoptera. For example, Deml & Dettner (1997) investigated the secondary chemistry of hemolymph and, in part, defensive secretions of several last-instar caterpillars from three moth families: emperor moths (Bombycoidea: Saturniidae), tussock moths (Noctuoidea: Lymantriidae), and prominents (Noctuoidea: Notodontidae). Defensive

secretions, if present, are discharged from specialized integument glands (Saturniidae: scoli; Lymantriidae: osmeteria). Many of the compounds identified by combined gas chromatography/mass spectrometry (phenolics, heterocycles, biogenic amines, aliphatics) were shown to exhibit toxic and/or irritant effects on a series of laboratory test-organisms, including micro-organisms, ants, and birds (summarized in Deml & Dettner 1997).

One particularly interesting finding was the striking similarity of the compound patterns in the three families. This was underlined recently (Deml & Nässig 2001) by the additional detection of several of the afore-mentioned compounds in the hemolymph of monkey moths (*Palirisa* sp.; Eupterotidae) which belong to the Bombycoidea just as the Saturniidae (Minet 1994; Lemaire & Minet 1999). In order to acquire more detailed information about this phenomenon, I determined semiquantitative data for all compounds identified from these larval body fluids (Deml & Dettner 1993, 1997; Deml 2001; Deml & Nässig 2001). Then I performed an analysis of resemblance of the chemical bouquets in order to assess the actual conformity of the caterpillars' chemical composition. Thereby, for the first time, all investigated caterpillar species were considered simultaneously in a multivariate approach, and hemolymph samples (four families) and secretions (Saturniidae/Lymantriidae) were distinguished.

Materials and methods

Caterpillar material. The last-instar caterpillars investigated (and the foodplants of the larvae used) were: (a) Saturniidae: *Saturnia pavonia* (*Crataegus monogyna*, *Prunus spinosa*), *S. pyri* (*C. monogyna*, *P. spinosa*), *Eupackardia calleta* (*Ligustrum vulgare*), *Attacus atlas* (*P. spinosa*); (b) Eupterotidae: *Palirisa* sp. (*L. vulgare*); (c) Lymantriidae: *Lymantria monacha* (*Larix decidua*), *L. dispar* (*Quercus robur*), *L. concolor* (*Rhododendron* sp.), *Euproctis chrysorrhoea* (*P. spinosa*); (d) Notodontidae: *Clostera curtula* (*Populus tremula*), *Notodonta ziczac* (*P. tremula*), *N. dromedarius* (*Betula alba*), *Pheosia tremula* (*P. tremula*), *Pterostoma palpina* (*P. tremula*), *Furcula bifida* (*P. tremula*), *Phalera bucephala* (*P. tremula*), *Stauropus fagi* (*Fagus sylvatica*).

Data analysis. Semiquantitative rank scores (intervals corresponding to: major/minor/trace/missing compounds, according to the total ion chromatograms) were determined for the hitherto identified secondary compounds of larval hemolymph (all species) and/or of defensive secretions (Saturniidae and Lymantriidae). Due to the small amounts of sample (of secretion, in particular) per individual caterpillar, in all cases the respective samples had been pooled from several larvae (Saturniidae: 3–5 larvae; Eupterotidae: 4 larvae; Lymantriidae: 4–8 larvae; Notodontidae: 3–5 larvae) before analysis. This resulted in elevated sum peaks of the compounds which could be better and more definitely evaluated. Altogether I detected 37 substances (2-pyrrolidone and GABA could not be chemically distinguished and were treated as one substance), all of which were aromatics, hetero- or alicycles, and aliphatic compounds (Table 1).

Subsequently the data were subjected to various clustering algorithms using the computer program NTSYS-PC 1.50 (Applied Biostatistics Inc.). At first, dissimilarity coefficients ('average taxonomic distances') were computed from a rectangular input

data matrix using the SIMINT program. 'Average taxonomic distances' represent Euclidean distances (shortest distances between two points in an *n*-dimensional space) divided by the number of characters (*n*) and are frequently used in numerical taxonomy as a means of measuring overall resemblance, particularly in case of large numbers of characters (Sneath & Sokal 1973: 124) or when values are compared between different studies having different numbers of characters (Abbott et al. 1985: 147). They were computed as follows:

$$d_{jk} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_{ij} - x_{ik})^2}$$

where d_{jk} = average taxonomic distance between objects *j* and *k*; *n* = number of characters; x_{ij}/x_{ik} = value (score) of the descriptor state that is observed for object *j*/*k* on variable (character) *i*. For comparison, average Manhattan distances (city block distances; also called Mean Character Differences, MCD) and average Canberra metrics were also computed. The former measure is also frequently used in numerical taxonomy and is a summation of the absolute differences between the objects on each of the variables taken in turn, divided by the number of characters (Sneath & Sokal 1973: 125; Abbott et al. 1985: 76, 147). The Canberra metric is a property solely of the two individuals/groups being compared in each pairwise comparison, is not affected by the range of the entire characters, and is sensitive to proportional rather than absolute differences (Sneath & Sokal 1973: 125). It is computed as the sum of absolute differences of the objects' values divided by the sum of the corresponding sums of the values. In the present case, distance values were adjusted for the number of characters through division by *n*.

Several clustering methods (for definitions see, for example, Sneath & Sokal 1973) were applied to the resulting distance matrices: average-based methods (UPGMA = unweighted pair-group method using arithmetic averages; WPGMA = weighted pair-group method using arithmetic averages; WPGMC = weighted pair-group method using centroid averages), the single-linkage as well as the complete-linkage methods, and the flexible clustering strategy (parameter $\beta = -0.25$). Cophenetic correlation coefficients (r_{CS}) were determined through a cophenetic value matrix. In the case of hemolymph samples, resulting multiple trees were combined by computing a consensus tree (strict method). For these, the corresponding consensus fork index (CI_c) was calculated.

Results

In most cases, the phenograms based on caterpillar hemolymph or gland secretions, which were obtained after applying the various dissimilarity coefficients and clustering methods, represented the corresponding distance matrices fairly satisfactorily. Distortion was low, as expressed by high cophenetic correlation coefficients (most $r_{CS} \geq 0.8$; Sneath & Sokal 1973) (Table 2). Also, the consensus trees computed from multiple trees in case of hemolymph were characterized by elevated consensus fork index (CI_c) values (Table 2), indicating a good representation of the original, single trees.

Compound	Saturniidae								Eup	Lymantriidae								Notodontidae							
	Saturnia pavonia		*Saturnia pyri*		*Attacus atlas*		*Eupackardia calleta*		*Palirisa sp.*	*Lymantria dispar*		*Lymantria monacha*		*Lymantria concolor*		*Euproctis chrysorrhoea*		*Clostera curtula*	*Notodonta ziczac*	*Notodonta dromedarius*	*Pheosia tremula*	*Pterostoma palpina*	*Furcula bifida*	*Phalera bucephala*	*Stauropus fagi*
	H	S	H	S	H	S	H	S	H	H	S	H	S	H	S	H	S	H	H	H	H	H	H	H	H
Toluene	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Xylene	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenol	+	+	+	+	+	-	?	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-
2-Cresol	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4-Cresol	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3,5-Dimethylphenol	+	+	+	+	?	?	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroquinone	+	?	+	+	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Veratrole	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzaldehyde	+	+	+	+	?	-	?	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-
Phenylacetaldehyde	‡	+	‡	‡	+	+	‡	‡	-	‡	+	‡	?	+	+	+	+	‡	-	-	-	-	-	-	-
Benzonitrile	+	+	+	‡	-	+	‡	‡	-	-	-	-	+	-	+	-	+	-	-	-	-	-	‡	-	-
Benzamide	-	-	+	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Phenetidine	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzylamine	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dopa	-	-	-	-	-	-	+	-	-																
Dopamine	-	-	-	-	?	+	+	-	-																
Noradrenalin	-	-	-	-	+	-	+	-	-																
Adrenalin	-	-	-	-	-	+	‡	‡	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nicotinic acid	-	-	-	-	-	-	?	?	-	‡	‡	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Nicotinamide	-	-	+	-	?	-	-	-	-	+	-	+	+	-	-	+	-	-	-	‡	-	-	‡	-	-
2-Pyrrolidone /	+	+	+	‡	+	‡	+	?	‡	‡	‡	+	+	+	+	+	+	-	-	-	-	-	-	-	-
γ-Aminobutyric acid (GABA)																									
1-Methyl-2-pyrrolidone	+	+	+	+	-	-	-	-	-	-	‡	-	+	-	+	-	+	-	-	-	-	-	-	-	-
Nicotine	-	-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Pyrazine	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Histamine	-	-	-	-	-	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarin	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	-	-	‡	‡	‡	‡	-																
2-Aminoethanol	-	-	-	-	-	?	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-(Methylamino)ethanol	-	-	-	-	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-(Dimethylamino)ethanol	-	-	-	+	‡	‡	?	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Choline	-	+	+	‡	+	‡	‡	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Aminoethyl acetate	?	?	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-(Methylamino)ethyl acetate	?	-	-	-	-	-	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-(Dimethylamino)ethyl acetate	-	?	-	-	-	-	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetylcholine	-	-	-	-	-	‡	‡	‡	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isopropyl myristate	-	+	+	+	-	?	?	-	-	-	?	+	+	+	+	+	?	-	-	-	-	-	-	-	-
Glycerol	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	++	??	-	+	+++	-	++	‡	++	-

Table 2. Cophenetic correlation coefficients (r_{CS}) and consensus fork indices (CI_c, plus corresponding numbers of trees; strict method) of the phenograms of larval gland secretions (S) or hemolymph (H), obtained after applying various dissimilarity coefficients and clustering methods.

	Dissimilarity coefficient	Average taxonomic distance		Manhattan distance		Canberra metrics	
Clustering method		r_{CS}	CI_c (trees)	r_{CS}	CI_c (trees)	r_{CS}	CI_c (trees)
UPGMA	H	0.80	0.87 (3)	0.84	0.87 (4)	0.95	0.87 (6)
	S	0.96	–	0.96	–	0.95	–
WPGMA	H	0.80	0.80 (3)	0.84	0.87 (4)	0.94	0.87 (6)
	S	0.96	–	0.96	–	0.94	–
WPGMC	H	0.80	0.87 (3)	0.83	0.87 (4)	0.94	0.87 (12)
	S	0.96	–	0.96	–	0.94	–
Single linkage	H	0.72	0.87 (6)	0.83	0.87 (6)	0.91	0.87 (6)
	S	0.95	–	0.95	–	0.93	–
Complete linkage	H	0.78	0.53 (7)	0.83	0.80 (8)	0.86	0.87 (9)
	S	0.95	–	0.96	–	0.94	–
Flexible clustering	H	0.64	0.53 (2)	0.61	0.73 (2)	0.84	0.93 (2)
	S	0.91	–	0.92	–	0.89	–

Most clustering methods using average taxonomic distances or Manhattan distances produced phenograms of the different hemolymph samples (Table 1) whose arrangements of the families were basically identical with the one illustrated in Fig. 1a. Here three groups were formed. The saturniids, *S. pavonia* and *S. pyri*, branch off first from the other species which in turn separate into one cluster of Notodontidae, and another one comprising Lymantriidae, Eupterotidae and the remaining Saturniidae. Distinguished from this, in the case of average taxonomic distances and single linkage (Fig. 1b), the four Saturniidae species branch off one by one, followed by the eupterotid species, then *L. dispar*, leaving one cluster each for the remaining Lymantriidae and the Notodontidae, respectively. Complete linkage as well as flexible clustering in combination with average taxonomic distances did not provide sufficient separation (one common origin of the clusters). In contrast, flexible clustering with Manhattan distances yielded a phenogram similar to UPGMA, but connected the eupterotid with the *S. pavonia*/*S. pyri* cluster. Since the r_{CS} and CI_c values, respectively, of these three pairings were rather low, these combinations are not considered as being convincing. Phenograms obtained with the Canberra metric (for example, Fig. 1c) differed from the corresponding ones obtained with average taxonomic distances mainly in that the notodontid spe-

Table 1. Occurrence of secondary compounds hitherto identified by GC-MS in hemolymph (H) and glandular secretions (S) from last-instar caterpillars of Saturniidae (4 spp.), Lymantriidae (4 spp.), Eupterotidae (1 sp.) and Notodontidae (8 spp.).
Above aromatics, center hetero- and alicyclic compounds, below aliphatic compounds. +++, main compound; ++, minor compound; +, trace; -, not detectable; ?, trace compounds with corresponding retention times but only incomplete EI mass spectra as compared with authentic chemicals; blank fields, analyses not performed. Prior to analysis, the respective samples had been pooled from 3–5 larvae (Saturniidae), 4 larvae (Eupterotidae), 4–8 larvae (Lymantriidae), or 3–5 larvae (Notodontidae).

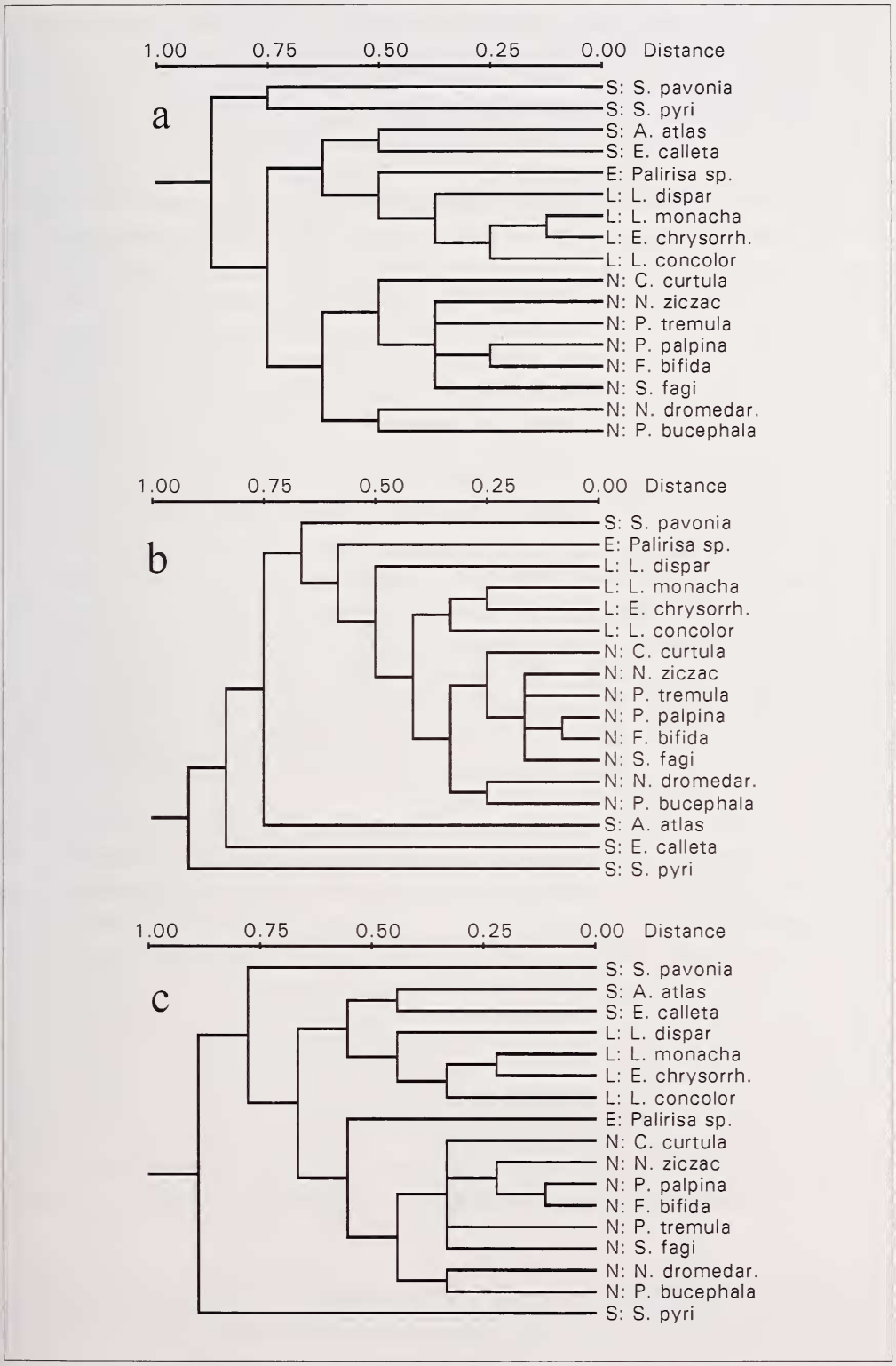
cies were arranged in a somewhat different order and the eupterotid species was regularly placed with the Notodontidae or, in case of flexible clustering, was grouped with *S. pavonia*/*S. pyri*. Thus, the main differences between the more relevant arrangements are the differing subdivision of the Saturniidae (one or two groups), on the one hand, and the position of the Eupterotidae, i.e., whether they are more closely combined with the Notodontidae, Lymantriidae, or Saturniidae, on the other hand.

Altogether three differing arrangements were also obtained after clustering the gland secretions of Lymantriidae and Saturniidae (Table 1, Fig. 2). Most clustering methods with average taxonomic distances as well as flexible clustering using the two other dissimilarity measures separated *S. pavonia* and *S. pyri* from *A. atlas* and *E. calleta*, yet in the reverse order as compared with the hemolymph. The Lymantriidae were positioned between these groups (Fig. 2a). Single linkage (Fig. 2b) and WPGMA, both using average taxonomic distances, resulted in a chain where *A. atlas* and *E. calleta* were separated as a cluster first, then *S. pyri*, *L. dispar*, *S. pavonia*, followed by the remaining lymantriids. In contrast, apart from flexible clustering, all clustering methods using Manhattan distances or Canberra metrics joined the Saturniidae together in an unbroken order (i.e., no lymantriid was intervening), either as a chain of single species (single linkage) or with *A. atlas* and *E. calleta* forming a pair (complete linkage, average-based methods; for example, Fig. 2c).

Discussion

The phenograms of the larval hemolymph samples revealed that the chemical patterns of the Lymantriidae and Notodontidae, respectively, yielded closed groupings of these families. This means that the bouquets of the hitherto identified chemical compounds in hemolymph are potentially suitable for characterizing families (and the species therein). However, the Saturniidae (the investigated species belong to the subfamily Saturniinae; Michener 1952) were split into two groups with most combinations of dissimilarity coefficients and clustering methods; the two groups corresponded to two tribes within the Saturniinae (Saturniini: *S. pavonia*/*S. pyri*; Attacini: *A. atlas*/*E. calleta*). The Saturniidae were located in a close arrangement only in case of average taxonomic distances and single linkage. The phenogram of this combination also showed a relative conformity of the taxonomic distribution of the substances with the currently supposed relationship of the four families. Lymantriidae and Notodontidae are placed in the superfamily Noctuoidea (Kitching & Rawlins 1999), whereas Saturniidae and Eupterotidae belong to the only distantly related superfamily Bombycoidea (Minet 1994; Lemaire & Minet 1999). However, the r_{CS} value obtained by this clustering combination was relatively low, indicating a comparably low correspondence of the dissimilarity matrix and the phenogram. The elongate growth of the single-linkage clusters ('chain-

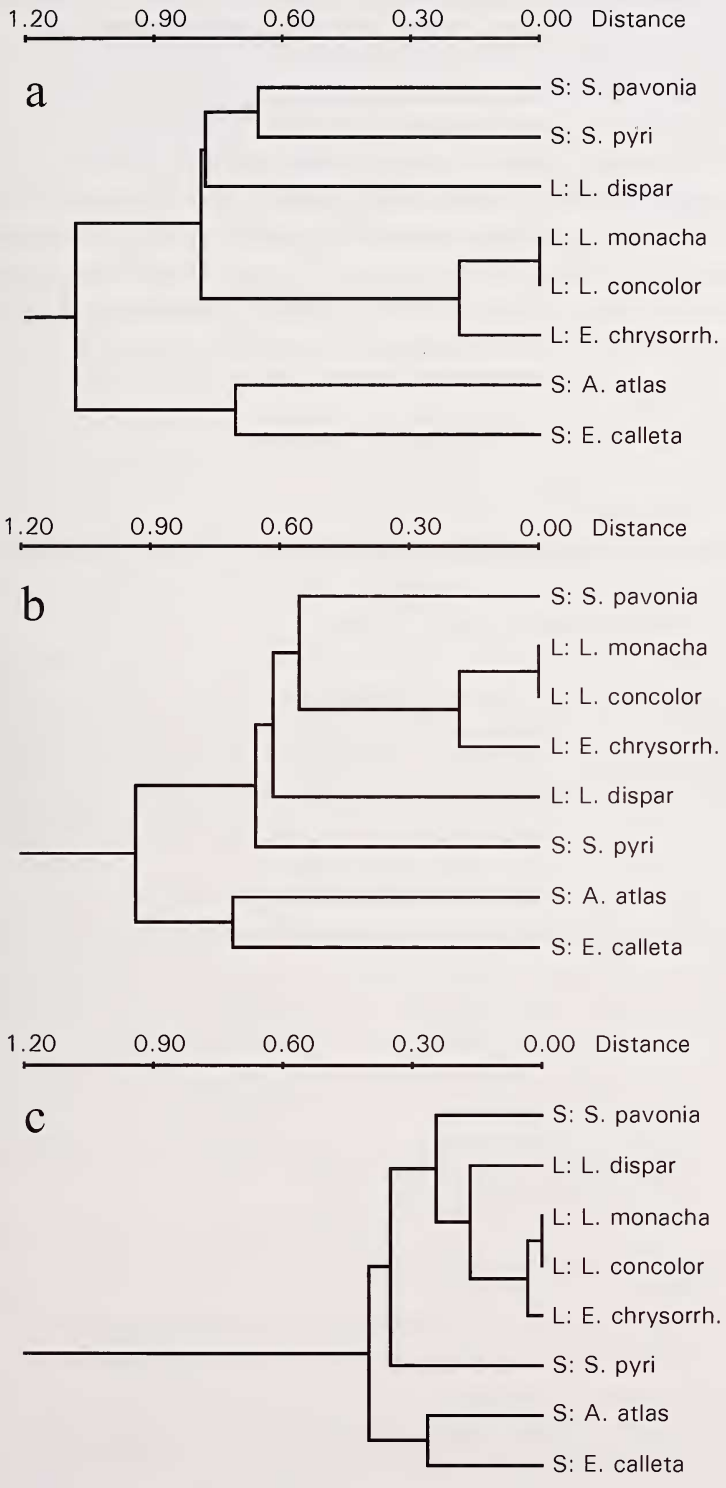
Fig. 1. Phenograms (strict consensus trees) of hemolymph from last-instar caterpillars of Saturniidae (S), Lymantriidae (L), Eupterotidae (E), and Notodontidae (N). Dissimilarity coefficients of the consensus trees were computed from an interval data matrix of amounts of 37 secondary compounds. a) average taxonomic distances, clustering by UPGMA, three resulting trees; b) average taxonomic distances, single linkage, six trees; c) Canberra metrics, UPGMA, six trees.


1.00
0.75
0.50
0.25
0.00
Distance
a
S: S. pavonia
S: S. pyri
S: A. atlas
S: E. calleta
E: Palirisa sp.
L: L. dispar
L: L. monacha
L: E. chrysorrh.
L: L. concolor
N: C. curtula
N: N. ziczac
N: P. tremula
N: P. palpina
N: F. bifida
N: S. fagi
N: N. dromedar.
N: P. bucephala
1.00
0.75
0.50
0.25
0.00
Distance
b
S: S. pavonia
E: Palirisa sp.
L: L. dispar
L: L. monacha
L: E. chrysorrh.
L: L. concolor
N: C. curtula
N: N. ziczac
N: P. tremula
N: P. palpina
N: F. bifida
N: S. fagi
N: N. dromedar.
N: P. bucephala
S: A. atlas
S: E. calleta
S: S. pyri
1.00
0.75
0.50
0.25
0.00
Distance
c
S: S. pavonia
S: A. atlas
S: E. calleta
L: L. dispar
L: L. monacha
L: E. chrysorrh.
L: L. concolor
E: Palirisa sp.
N: C. curtula
N: N. ziczac
N: P. palpina
N: F. bifida
N: P. tremula
N: S. fagi
N: N. dromedar.
N: P. bucephala
S: S. pyri

ing') which occurs particularly when there are a number of equidistant or near-equidistant points (Sneath & Sokal 1973), also indicates that this 'nearest neighbour' method might not be optimal for the present analysis. The phenograms generated by average-based methods (such as UPGMA) using average taxonomic distances or Manhattan distances as well as the one resulting from the 'farthest neighbour' method (complete linkage) using Manhattan distances were basically identical (the same was found with the corresponding clusterings with the Canberra metrics). One may therefore conclude that the absolute differences of the original data-matrix values are relatively small. The range of almost all distance values per species in the distance matrix was <0.3 (most differences even <0.2; corresponding distance values between 0.0 and 0.9) with average taxonomic distances, <0.2 with Manhattan distances (corresponding distance values: 0.0–0.65), and <0.55 with Canberra metrics (corresponding distance values: 0.0–0.6). There were many identical distance values in each matrix. Therefore the phenograms using Canberra metrics, although having the highest cophenetic correlation coefficients, might rather reflect an overemphasis of proportional differences between the original values by this dissimilarity coefficient. This might be the reason for the altered structure of the Notodontidae and their 'wrong' grouping with the species of Eupterotidae, in comparison with most unique phenograms made by means of the two other dissimilarity coefficients. However, for the moment it cannot be excluded that any observed conformity of resemblance of hemolymph chemistry, on the one hand, and taxonomy, on the other hand, is conditional on mere accident. Results from such phenetic analyses *per se* need not have any connection with phylogenetic relations between families or species, as long as no hemolymph compounds have been explained as synapomorphies. One should keep in mind that all the 37 compounds used in this study were scored for multivariate analysis in an identical manner, irrespective of their structural complexity and state of derivation.

It appears more difficult to interpret the results from the cluster analyses of the exocrine secretions of Lymantriidae and Saturniidae. The cophenetic correlation coefficients for all phenograms obtained are very high. The three arrangements shown in Fig. 2 particularly differ in the position of *S. pavonia* and *S. pyri*. While in Fig. 2a these species form a group which is very distant from the other saturniids, *A. atlas* and *E. calleta* ('beyond' the Lymantriidae), in Fig. 2b *S. pyri* has changed sides, and in Fig. 2c *S. pavonia* has done so, too. The phenogram in Fig. 2c (most methods using Canberra metrics or Manhattan distances) shows a considerably larger resemblance of the secretions than in the two other phenograms which indicates that not only the sums of the objects' values (scores) are small but also their differences. The distances in Fig. 2b are generally only somewhat smaller than in Fig. 2a which is probably simply caused by the 'space dilating' effect of average-linkage clustering methods (UPGMA etc.; Fig. 2a) and the corresponding 'space contracting' properties of single-linkage, respectively,

Fig. 2. Phenograms of gland secretions from last-instar caterpillars of Saturniidae (S) and Lymantriidae (L). Dissimilarity coefficients of the trees were computed from an interval data matrix of amounts of 37 secondary compounds. a) average taxonomic distances, clustering by UPGMA; b) average taxonomic distances, single linkage; c) Canberra metrics, UPGMA.


1.20
0.90
0.60
0.30
0.00
Distance
a
S: S. pavonia
S: S. pyri
L: L. dispar
L: L. monacha
L: L. concolor
L: E. chrysorrh.
S: A. atlas
S: E. calleta
1.20
0.90
0.60
0.30
0.00
Distance
b
S: S. pavonia
L: L. monacha
L: L. concolor
L: E. chrysorrh.
L: L. dispar
S: S. pyri
S: A. atlas
S: E. calleta
1.20
0.90
0.60
0.30
0.00
Distance
c
S: S. pavonia
L: L. dispar
L: L. monacha
L: L. concolor
L: E. chrysorrh.
S: S. pyri
S: A. atlas
S: E. calleta

where no average distance-values of clusters but only the nearest neighbours are considered. Considering such shortcomings of the single-linkage method, the 'intermediate' phenogram obtained by this method as well as by WPGMA using average taxonomic distances (Fig. 2b) seems to be the least trustworthy. However, Abbott et al. (1985) recommend to use just the single-linkage method, at least above the species level, because the corresponding diagrams would reveal the full diversity of clusters, chains, and outliers, whereas group-average methods (UPGMA, WPGMA) only reveal a quick look at a variation pattern, but at the expense of precision and loss of information. Furthermore, only single linkage always places an item in the same cluster as its nearest neighbour in the geometric model, whereas in methods such as UPGMA the nearest neighbours may be led into separate clusters due to space dilation.

The other two, extreme phenograms of the gland secretions suggest two ideas, but it cannot be decided at present which one is 'correct', or whether none or even both are 'correct'. First, the phenograms obtained by 10 clustering-method combinations using Canberra metrics or Manhattan distances (Fig. 2c) separate the two families in so far as the concerned species per family are arranged in an order – though not in two separate clusters – reflecting their taxonomic affiliations (no intervening member of the other family, in each case). Secondly, although these secretions are discharged by morphologically very different glands (Deml & Dettner 1997), the position of *L. dispar* and the other Lymantriidae in between the saturniids in the phenograms produced by six method combinations (most clusterings using average taxonomic distances as well as flexible clustering using the other two dissimilarity coefficients; Fig. 2a) could point at different adjustments of the ecological function of the secretions of the two families which is assumed to be chemical defence against enemies. For example, the secretions of *S. pavonia*, *S. pyri*, and the Lymantriidae as well as several compounds therein are effectively deterring ants (Deml & Dettner 1993, 1997; Aldrich et al. 1997). In contrast, *E. calleta* and *A. atlas* (both species are not Palaearctic) additionally discharge large amounts of biogenic amines (e.g.: histamine, acetylcholine) which are assumed to be effective against vertebrates (Deml & Dettner 1997). Combinations of high titres of especially ant-deterring substances (benzaldehyde, phenylacetaldehyde, 2-pyrrolidone/GABA, 1-methyl-2-pyrrolidone, nicotine; Table 1) in the scolus secretions of *S. pavonia* and *S. pyri* and in the osmeterial secretion of the Lymantriidae, in particular of *L. dispar*, explain the close arrangement of these species in the phenograms, while the weighting of the cyclic compounds (second section in Table 1) obviously particularly influences the species' relative order in Figs. 2a and 2b.

The three phenograms of the larval secretions, in particular, distinctly illustrate that it can be very informative to compare various combinations of selected distance measures and clustering algorithms. As could be shown, alternative but equally plausible conclusions may be drawn from identical data material when subjected to different methods of data analysis. A mistake when formulating one-sided hypotheses relying on only a single, arbitrary method can be avoided by such comparisons. However, one must be aware that such data analyses provoking new thoughts do not represent the final point of an investigation, but only an intermediate step which may guide the further procedure into a completely different direction.

Finally, a high relative similarity of the chemical bouquets of most secretions is obvious. In most species of Saturniidae and Lymantriidae studied thus far, the patterns of hemolymph compounds are also quite similar to those of the secretions (Deml & Dettner 1997). Superficially, this might be explained as uniformity caused by equal larval food (leaves from few species of deciduous trees and shrubs used in rearing). However, as stated earlier (Deml & Dettner 1993, 1997), most of the substances found in Lymantriidae and Saturniidae are probably biosynthesized by the caterpillars. Analyses of various foodplant samples and comparisons to the larval components revealed only single, occasional cases of correspondence (e.g., benzaldehyde, nicotinic acid). Furthermore, chemicals such as acetylcholine, histamine, or benzonitrile, whose synthesis requires many metabolic steps, are unlikely to represent merely detoxication or degradation products of toxic plant substances. It is possible, yet has not been tested, that the substances are produced through identical metabolic pathways. If this were the case, biosynthetic parallels in these moth families could point at certain basic and common metabolic processes within them and possibly also within a larger part of the Macrolepidoptera. However, more data on the origin of the compounds (sequestration from food or biosynthesis by larvae) and analyses of additional relevant species and moth families are required to address this question conclusively.

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